تأثير الملوحة في محتويات كل من البرولين والبيتائين في نبات البنج الذهبي

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الملخص

تم إخضاع ثلاث مجموعات من نبات البنج الذهبي *Hyoscyamus aureus* إلى ثلاث درجات مختلفة من الملوحة، وأجريت الدراسة لتوضيح العلاقة بين المحتويات الشاردية غير العضوية والمحتويات من البيتائين والبرولين، ويعدُّ كلاهما جزيئات واقية حلولياً للنبات. أظهرت النتائج الواردة في هذا البحث، أنه كلما ازداد متوسط الوزن الجاف والمحتوى للمجموع الشاردي (K^+,Na^+) وكلما انخفضت النسبة الشاردية (Na^+/Ca^{2+})، ارتفعت محتويات البرولين في الأوراق والجذور. إن تراكم البرولين يعدُّ استجابة لنقص الماء الحاصل في النباتات، ويعدُّ أيضاً استجابة ليس فقط لتراكم شوارد Na^+ وإنما أيضاً لتراكم شوارد K^+ وتأثير شوارد Ca^{2+} الواقية.

إن زيادة محتويات البيتائين في الأوراق والجذور لا يعتمد فقط على تركيز شواردNa^+ ولكن يعتمد أيضاً على تركيز الجزيئات الشاردية الكبيرة المغذية Macronutrient ionic concentration، وتظهر هذه الاستجابة حتى مع حصول تأخر محدد. ويتبين من خلال النتائج التي تم الحصول عليها أن تراكم البيتائين في نبات *Hyoscyamus aureus* لا يعدُّ مقياساً Parameter موثوقاً به لكشف الشدة الملحية Salinity Stress وإنما يعبّر عنها من خلال زيادة محتوى البرولين الحر والذي يزداد وفقاً لتأثيرات الملوحة Salinity conditions

الكلمات المفتاحية: برولين، بيتائين، الملوحة، البنج الذهبي.

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Effect of salinity in free proline and betaine contents in *Hyoscyamus aureus* plants

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Abstract

Hyoscyamus aureus **plants were stressed with three different degrees of salinity in order to study relationships between inorganic ion contents and the contents of betaine and proline, both molecules being considered as plant osmoprotectives.**

Our results demonstrated that the greater the dry weight average and the (Na^+ + K^+) content and the lower the (Na^+ / $Ca2^+$) ratio, the higher the proline contents in leaves and roots. Proline accumulation is considered as a response to water deficit and a response against not only Na^+ ions but also K^+ ions accumulation and Ca^{2+} ions protector effect.

Betaine contents in leaves and roots were not only Na^+ concentration dependent but also depended on macronutrient ionic concentration, and this response showed up with certain lag. We can conclude from our results that in *Hyoscyamus aureus* plants, betaine accumulation is not a reliable parameter to detect salinity stress, but free proline content increases according to the salinity conditions.

Keywords: proline, betaine, salinity, *Hyoscyamus aureus.*

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1-Introduction:

Plants have developed different ways to survive under high salt concentration conditions.

The osmotolerance may be a means to protect sensitive organs (such as young developing leaves) from salinity, by a discontinous salt accumulation within the plant. Inorganic ions may also be stored in vacuoles. In response to the salinity a large number of species, increase their intracellular soluble components and accumulate different small organic molecules, mainly betaines and/or amino acids such as proline all of which are considered as plant osmoprotectives.

Betaine has been considered a fairly stable metabolism product, but besides its possible role as a methyl Donor, little is known about its function but it may represent up to 20% of the total plant nitrogen in some healthy young plants grown in the field.



Proline is another organic compound that has been reported to accumulate in glycophytes and halophytes Several researches have found specific relationships between the cellular inorganic ions composition and proline accumulation. In halophytes a positive relationships between proline accumulation and the cell Na^+ and Cl^- content was reported. The proline accumulation metabolic mechanism has been studied, and the resuts showed that it is due to the stimulation of its synthesis from glutamic acid. Proline oxidation inhibition also contributes to its accumulation as well as its impaired utilization in the protein syntheses.

2-Material and methods:

2-1 Culture conditions

Seeds of *Hyoscyamus aureus* plants (obtained from Idebl) were sown in equal size flower pots containing a mixture of sand and vermiculite. Pots

were moistened with the same amount of deionised water three times a week, when the germination began, the Plantules were divided into four groups; the first was rinsed with Arnon and Stoud solution and used as a Control, the second was rinsed with the same solution, but with 20 mM NaCl (20 mM NaCl plants), the third with 40 mM NaCl added to it (40 mM NaCl plants) and the fourth with 80 mM NaCl (80 mM NaCl plants). The plants were harvested after 12, 14, 16, 17, 18, 19 weeks of the treatment. In each sample three different plants were harvested and for each plant we analysed leaves and roots twice. Thus, the total number of analysis for each sample was six. Leaves and roots were sampled separately.

The following measurements and analyses were carried out: measurement of growth (dry weight), ion determination, (Na^+, K^+, Ca^{2+}), proline, betaine.



2-2 Average dry weight

The average dry weight was calculated for leaves and roots from their dry and fresh weights.

Dry weight determination were made by heating leaves and root separately at 60°C for 48 hours and then to constant weigth.





2-3-Ion determination

Na^+, k^+ and Ca^{2+} determination were made by emission spectroscopy for plasma ICP in a Mixter spectrometer multichannel sequenical pf plasma ICPJY 70 plus (Vindel, 1985).

2-4 Proline determination

The free proline extraction and purification was made according to Bates (1973) and the spectrophotometryc determination using.by Hitachi U 2000 spectrophotometer, as according to the same author Bates (1973).

2-5 Betaine determination

The total betaine extraction, purification and determination was carried out according to Gorhan et all. using an HPLC, LKB Broma, with a partisil 10SCX (425 x 2)mm columm and 1.25 M NH4HgPO4 (pH=2.7) in methanol as solvent.

3-RESULTS AND DISCCUSSION

3-1 Average dry weight

The average dry weight of leaves and roots in the control plants increased continuously during the experiment, (Figure 1). The average dry weight in stressed plants also increased during the experiment and was higher the greater the salinity conditions and the longer stress time. These results were due not only to innert substances accumulation but also to the water deficit in stressed plants tissues.

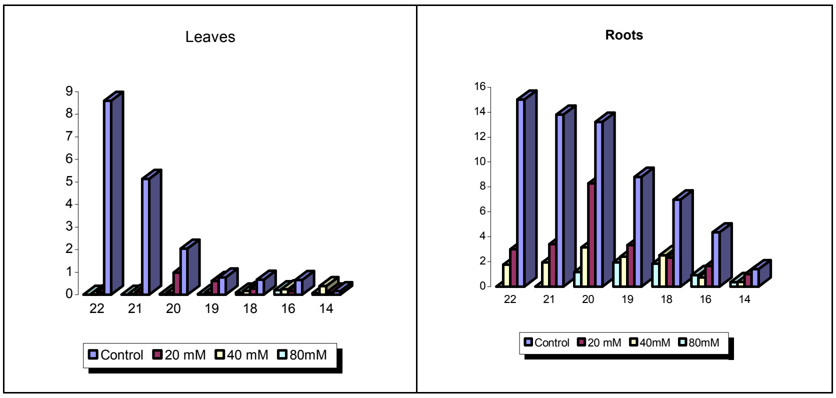


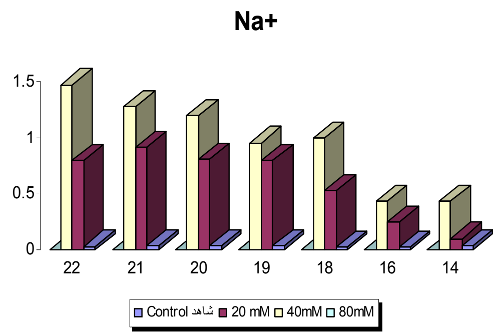
Figure 1. Dry weight (DW) expressed in g.Data are the average of six analyses. Weeks : weeks of treatment

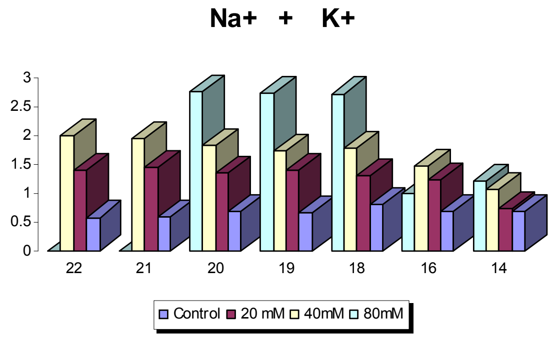
3-2-Univalent ions content

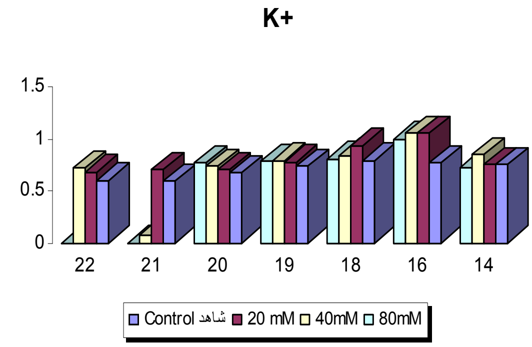
The contents of K+ in roots and leaves of control plants remained relatively constant and in the interval considered as normal during the first 17 weeks of growth and went down at the end of the experiment (tab.1and2), probably due to inert substances accumulation in plants . Roots and leaves of control plants had a very low content of Na+ and tot M (Na+ + K+) contents were very similar to K+ (tab. 1and2) .The roots of stressed plant had K+ contents lower the higher salinity conditions of growth and the longer the time of stress. Na+ contents of 20 mM plants roots increased during the experiment but 40 mM plants roots Na+ contents increased only until the 18^{th} week of growth and then decreased, until the end of the experience; and finally Na+ contents of 80 mM plants roots reached very high levels during the first 14 weeks of growth decreasing thereafter until the end of the experiment. A high content of Na+ inhibited the root growth and led to necrosis and loss of young roots tissues in no-halophytic plants (Carmer et al., 1986). Tot M contents in roots of stressed plants were similar to content of Na+ .The results showed that in our experimental conditions, the limit for normal life of root of *Hyoscyamus aureus* had a content of totM 2 times higher than the roots of control plants. Roots of 40 mM and 80 mM plants reached this limit after 18 weeks and 14 weeks of growth respectively, then they underwent necrosis and death. The content of K+ in leaves of stressed plants were slightly higher than in leaves of control plants, specially in those of 40 mM plants. These results agree with Grenway et all (1966), who reported an increase in contents of K+ and Na+ in leaves of salt sensitive plants. Contents of Na+ and Tot M ions were notably higher in the leaves of plants grown in salinity conditions than in the leaves of control plants and were higher the greater the salinity conditions and time of stress. Our results showed that, generally, when the Na+ contents in leaves reached 0.7 meq/g DW , their content of univalent ions was tow times higher than in control plants leaves, this high content is the limit of viability on *Hyoscamus aureus* leave-cells.

Table 1.K+,Na+ and Tot M (Na+,K+) content in roots of treated and control plants, expressed in meq/g DW . Data are the average of six analyses. Weeks : wekks of treatment.

Weeks	Control	20 mM	40mM	80mM
		Na^+		
14	0.033±0.001	0.098±0.004	0.435±0.001	0.594±0.010
16	0.019±0.000	0.249±0.020	0.431±0.017	1.113±0.087
18	0.025±0.007	0.527±0.031	1.000±0.070	2.006±0.205
19	0.032±0.015	0.799±0.062	0.956±0.085	2.000±0.101
20	0.032±0.030	0.808±0.082	1.196±0.099	2.008±0.112
21	0.031±0.016	0.911±0.077	1.275±0.073	-
22	0.029±0.033	0.800±0.068	1.471±0.065	-
		K^+		
14	0.753±0.054	0.751±0.049	0.842±0.060	0.728±0.044
16	0.765±0.039	1.055±0.095	1.049±0.084	1.000±0.080
18	0.793±0.063	0.933±0.065	0.838±.0.033	0.810±0.069
19	0.731±0.059	0.775±0.065	0.788±0.051	0.781±0.068
20	0.671±0.043	0.700±0.039	0.732±0.047	0.769±0.080
21	0.593±0.037	0.700±0.045	0.0768±0.057	-
22	0.599±0.039	0.681±0.050	0.715±0.040	-
		Na^+ + K^+		
14	0.680±0.054	0.740±0.073	1.077±0.060	1.222±0.095
16	0.690±0.039	1.230±0.104	1.480±0.084	1.000±0.273
18	0.800±0.063	1.320±0.127	1.779±.0.100	2.727±0.069
19	0.670±0.059	1.400±0.140	1.729±0.149	2.729±0.207
20	0.698±0.043	1.362±0.155	1.835±0.164	2.756±0.180
21	0.600±0.070	1.460±0.100	1.956±0.187	-
22	0.582±0.039	1.400±0.117	1.999±0.284	-







Fig(2):K+,Na+ and Tot M (Na+,K+) content in roots of treated and control plants, expressed in meq/g DW. Data are the average of six analyses.

Table 2. K+,Na+ and Tot M (Na+,K+) content in leaves of treated and control plants, expressed in meq/g DW . Data are the average of six analyses. Weeks : wekks of treatment.

Weeks	Control	20mM	40mM	80mM
	Na^+			
14	0.133±0.017	0.514±0.063	0.1.218±0.105	1.980±0.179
16	0.243±0.025	0.644±0.077	1.491±0.115	1.577±0.154
18	0.190±0.007	0.851±0.071	1.856±0.160	1.193±0.205
19	0.290±0.045	0.947±0.035	1.701±0.153	0.860±0.085
20	0.284±0.039	0.997±0.082	1.650±0.145	0.454±0.053
21	0.256±0.022	1.253±0.099	1.282±0.100	-
22	0.260±0.031	1.800±0.110	1.270±0.113	-
	K^+			
14	1.058±0.093	0.851±0.089	0.542±0.060	0.558±0.081
16	0.815±0.059	0.591±0.045	0.365±0.084	0.266±0.080
18	0.752±0.053	0.290±0.025	0.175±.0.033	0.181±0.019
19	0.705±0.069	0.210±0.017	0.155±0.051	0.125±0.008
20	0.705±0.073	0.178±0.033	0.157±0.047	0.154±0.080
21	0.878±0.077	0.169±0.031	0.159±0.057	-
22	0.845±0.089	0.145±0.011	0.115±0.040	-
	Na^+ + K+			
14	1.187±0.102	1.310±0.124	1.805±0.169	2.459±0.263
16	0.982±0.091	1.114±0.114	1.765±0.154	1.835±0.165
18	0.916±0.063	1.053±0.107	2.015±.0.180	1.244±0.146
19	0.860±0.059	1.035±0.095	1.789±0.159	0.857±0.096
20	0.801±0.073	1.098±0.088	1.715±0.160	0.569±0.064
21	0.927±0.079	1.311±0.104	1.351±0.121	-
22	1.014±0.100	1.319±0.117	1.275±0.110	-

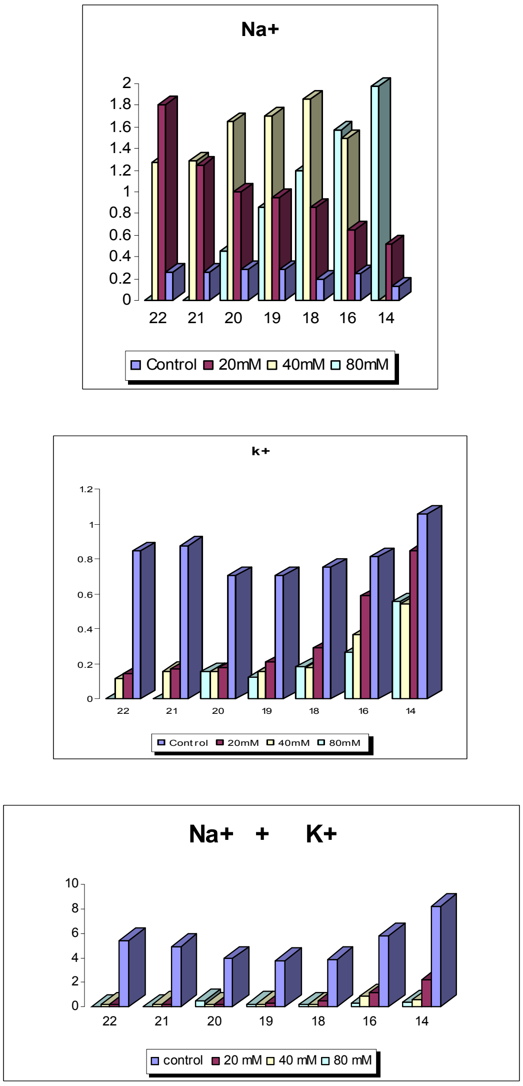
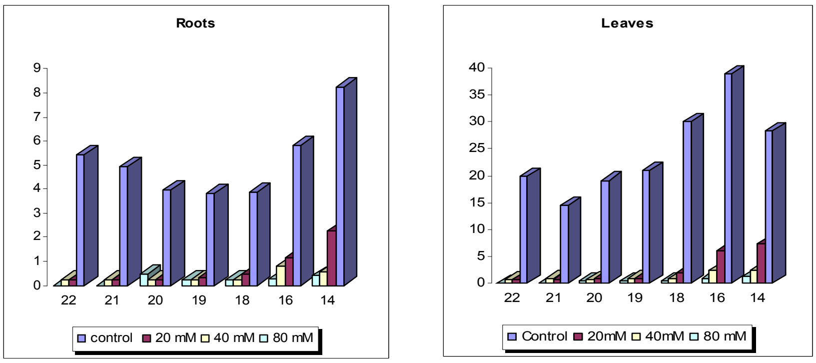


Figure 3. Na+, K+ content in leaves of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment

Table3.K+, Na+ and Tot M (Na+,K+) content in leaves of treated and control plants, expressed in meq/g DW . Data are the average of six analyses

Weeks	Leaves			
	Control	20mM	40mM	80mM
14	28.39	7.38	2.33	1.33
16	38.92	6.11	2.30	0.96
18	30.13	1.85	0.80	0.46
19	21.03	0.96	0.80	0.44
20	19.13	0.85	0.65	0.42
21	14.50	0.74	0.85	-
22	19.96	0.72	0.56	-
	Roots			
14	8.23	2.26	0.58	0.39
16	5.81	1.15	0.82	0.26
18	3.89	0.48	0.20	0.21
19	3.80	0.33	0.22	0.22
20	3.95	0.20	0.20	0.44
21	4.95	0.24	0.23	-
22	5.44	0.22	0.20	-



Fig(4):K+/Na+ contents ratio in leaves and roots

On the other hand Jeschke 1979 showed that senescence is started when the K+/Na+ ratio is lower than 1(Table 3). Our results showed that

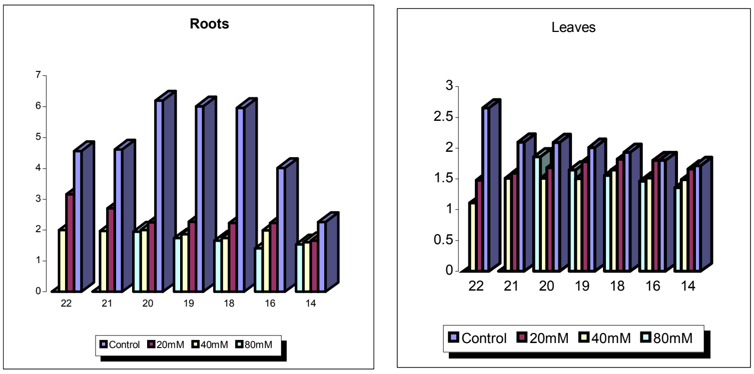
K^+/Na^+ ratio in leaves of 20 mM, 40 mM and 80 mM plants were lower than 1 at 18, 19 and 16 weeks respectively , and the roots of these plants at 18 and 16 respectively. We infer than these plants suffered stronger degarding processes the higher the salinity conditions were.

Ca^{2+} content:

The Ca^{2+} content in roots of plants control increased until the 20th week and then decreased until the end of experiment, probably due to necrosis and loss of the youngparts of roots (Table 4) .The leavels of Ca^{2+} in the roots of stressed plants of *Hyoscyamus aureus* increased during the course of the experiment, but they were lower than the levels in roots of control plants and were lower the higher the salinity conditions of grown, and the longer the time of stress. The Na^+/Ca^{2+} ratio (Table 5) was low and relatively constant in the roots of control plants during the course of the experiment, but this ratio increased and then decreased in the roots of 20mM and 40 mM plants and decreased continuously in the roots of 80 mM plants Whittington and Smith 1992 considered that high Na^+/Ca^{2+} ratios damage semipermeability and permit Na^+ and K^+ uptake but lower Na^+/Ca^{2+} ratio maintain semipermeability. We must not consider the decrease of Na^+/Ca^{2+} ratio in the stressed roots as a protective mechanism, due to necrosis and loss of young parts of roots, because Ca^{2+} a linked to the older part of plants. The levels Ca^{2+} in the leaves of *Digitalis purpuria* control plants were sufficient and increased slightly during the course of the experiment due to the increase in the number, of old leaves with respect to young ones. On the contrary, leaves of 20 mM and 40 mM plants increased their contents in Ca^{2+} for 18 weeks and then decreased due loss of old leaves rich in Ca^{2+}. The content of Ca^{2+} in leaves of 80 mM plants increased during the experiment because their leaves were always in senescence. The Na^+/Ca^{2+} ratio was low in the leaves of control plants, but very high in leaves of stressed plants, showing the damage in the latter.

Table 4. Ca2+ values in leaves and roots expressed in meq/g Dw. Data are the average of six analysis. Weeks: weeks of treatment .

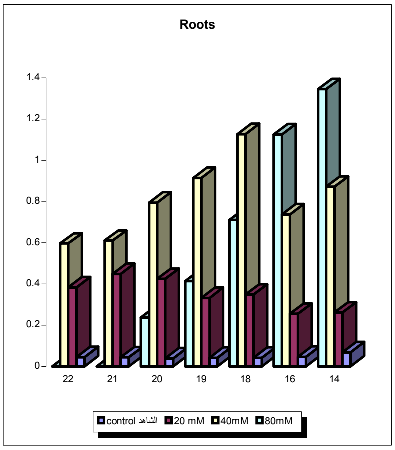
Weeks	Leaves			
	Control	20mM	40mM	80mM
14	1.719±0.143	1.662±0.150	1.490±0.127	1.364±0.114
16	1.8011±0.158	1.801±0.144	1.521±0.142	1.468±0.162
18	1.934±0.166	1.826±0.169	1.644±0.190	1.560±0.157
19	2.014±0.191	1.786±0.138	1.510±0.128	1.647±0.166
20	2.094±0.183	1.684±0.162	1.513±0.161	1.861±0.171
21	2.101±0.204	1.597±0.128	1.513±0.129	-
22	2.655±0.217	1.481±0.133	1.109±0.150	-
Roots				
14	2.255±0.238	1.662±0.089	1.606±0.153	1.537±0.122
16	4.014±0.310	2.229±0.132	1.992±0.167	1.413±0.130
18	5.958±0.447	2.230±0.137	1.744±.0.143	1.656±0.164
19	6.010±0.384	2.273±0.129	1.870±0.129	1.737±0.164
20	6.198±0.362	2.248±0.200	1.998±0.166	1.951±0.129
21	4.619±0.299	2.705±0.211	1.966±0.169	-
22	4.563±0.273	3.171±0.197	1.997±0.174	-

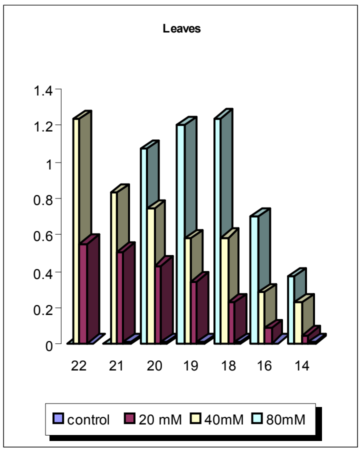


Fig(5):Ca2+ values in leaves and roots expressed in meq/g Dw. Data are the average of six analysis

Table 5. Na+/ Ca2+ contents ratio in leaves and roots

Weeks	Leaves			
	Control	20mM	40 mM	80mM
14	0.012	0.052	0.229	0.372
16	0.008	0.091	0.283	0.699
18	0.011	0.234	0.585	1.240
19	0.014	0.348	0.579	1.198
20	0.015	0.432	0.745	1.071
21	0.016	0.511	0.832	-
22	0.008	0.553	1.233	-
Roots				
14	0.068	0.261	0.872	1.345
16	0.046	0.255	0.738	1.126
18	0.042	0.348	1.127	0.712
19	0.042	0.332	0.915	0.414
20	0.039	0.424	0.795	0.237
21	0.044	0.448	0.610	-
22	0.045	0.383	0.596	-





Fig(6):Na+/ Ca2+ contents ratio in leaves and roots

3-3 Free proline contents

The free proline contents in roots and leaves of control plants remained relatively low and constant during the first 16 weeks of growth and rised at the end of the experiment (Table 6).

In general stressed plants had a higher free proline contents in roots and leaves than control plants. The free proline contents in roots and leaves of 20 mM plants remained constant during the first 16 weeks of growth and then clearly increased; at this moment also the K^+/ Na^+ ratio became lower than 1, Jeschke found that this value (K^+/ Na^+ ratio) is a senescence mechanism trigger. The proline content, thus, increased at that moment (16 weeks of growth) when also the Na^+/ Ca^{2+} ratio became higher than 0.35. Whinttihgton et all. found that large Na^+/ Ca^{2+}ratios damage the membrane semipermeability and produce a metabolic change.

The contents of free proline in roots and leaves of 40 mM plants was higher than in 20 mM plants, and was the higher in roots and leaves of 80 mM plants where it continued to increase until the death of plants.

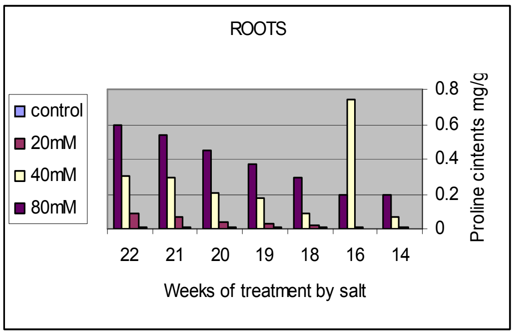
A note worthy fact was that free proline contents increased in a similar way to Na^+ + K^+ content, specially in roots of 40 and 80 mM plants, where Na^+ + K^+ content exceeded 2. weimberg found this level as a proline synthesis activator.

In the present experiment the roots had a high free proline content. These results do not agree either with Weimberg (1986) or our own earlier ones. In our previous paper we found proline was specially accumulated in leaves. The reason for this unusual accumulation of free proline in roots would be the high K^+ content in *Hyoscyamus aureus* roots in this experiment, In fact, Weimberg et al found that free proline accumulation was triggered specially by K^+ contents, not by other ion contents.

The free proline contents in roots and leaves of *Hyoscyamus aureus* plants was related to dry weight average. Table 1 shows that free proline contents in leaves and roots of control and 20 mM plants was low but increased during the experiment and when the average of dry weight became 20% (16 weeks of growth), the increase of free proline content was apparent. The roots and leaves of 40 and 80 Mm plants had a very high free proline contents and this average dry weights (Figure 7) were higher than 20%. Our results agree with Stewart, who proved that proline synthesis follows the ABA increase due to water deficit.

Table 6. Proline content of treated and control plants, expressed in mg/g DW. Data are the average of six analyses

	Control	20mM	40 mM	80 mM
Week	**LEAVES**			
14	0.004 ± 0.000	0.0160 ± 0.005	0.154 ± 0.034	0.191 ± 0.063
16	0.005 ± 0.000	0.015 ± 0.002	0.166 ± 0.041	0.204 ± 0.070
18	0.005 ± 0.000	0.016 ± 0.003	0.168 ± 0.037	0.228 ± 0.083
19	0.013 ± 0.001	0.026 ± 0.004	0.178 ± 0.070	0.227 ± 0.094
20	0.022 ± 0.001	0.082 ± 0.010	0.181 ± 0.059	0.303 ± 0.078
21	0.034 ±0.002	0.098±0.021	0.0190±0.077	0.302 ±0.088
22	0.042 ± 0.005	0.101 ± 0.044	0.209±0.069	0.190 ±0.071
Week	**ROOTS**			
14	0.003 ± 0.000	0.006 ± 0.001	0.069 ± 0.011	0.199 ± 0.031
16	0.003 ± 0.000	0.006 ± 0.001	0.074 ± 0.009	0.199 ± 0.044
18	0.005 ± 0.000	0.017 ± 0.003	0.172 ± 0.013	0.294 ± 0.032
19	0.005 ± 0.001	0.025 ± 0.002	0.209 ± 0.026	0.375 ± 0.080
20	0.007 ± 0.001	0.042 ± 0.007	0.181 ± 0.048	0.444 ± 0.096
21	0.012 ±0.000	0.071 ±0.007	0. 291±0.059	0.541 ±0.121
22	0.014 ± 0.000	0.088 ± 0.010	0.302±0.081	0.599 ±0.191



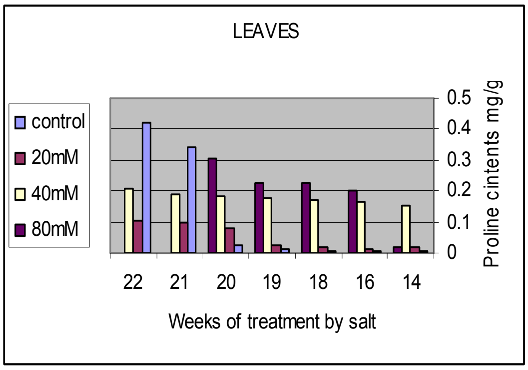


Fig (7): Proline content of treated and control plants in leaves& roots, expressed in mg/g DW. Data are the average of six analyses

3-4 Betaine contents

The betaine contents in leaves of control and 20 mM plants, increased during the experiment, but remained always at low levels. (Table 7). The betaine contents in 40 mM plant leaves, after 12 weeks of growth, was higher than leaves of control and 20 mM plants and increased until the end of the experiment, becoming four times greater than the contents of control plant leaves.

The leaves of 80 mM plants had betaine contents significantly higher than leaves of 40 mM plants during the first 12 weeks of growth and increased

during the following fourth weeks, thereafter decreased until the death of the plants.

The plants grown in different salinity conditions showed different behavior in our experiments. Leaves stressed with low salt concentrations (20 mM NaCl) did not respond to stress, Koheil et al.found, in *Atriplex semibaccatta*, that betaine synthesis depends not only on Na^+ concentration but also on macronutrient ionic concentrations. In this work we have measured only the Na^+, K^+ and Ca^{2+} contents, but since K^+ and Ca^{2+} constitute the majority of cations in the nutritive medium, we have considered the $Na^+ + K^+ + Ca^{2+}$ contents as an indicator of the ionic concentration in plant cells.

The $Na^+ + K^+ + Ca^{2+}$ contents in leaves of 20 mM plants (Figure 2) was at first slightly higher and then lower than the control plant leaves contents. According to Koheil et all. the ionic concentration in 20 mM plants leaves was not enough to activate the betaine synthesis. On the contrary betaine contents in leaves of 20 mM plants (intermediate or low salinity) increased continuously, and the Figure 2 shows that $Na^+ + K^+ + Ca^{2+}$contents was significantly high during the first 19 weeks of growth and then became lower than in leaves of control plants, but betaine contents rised (Table 7).

These results agree with Weimbergs who found that betaine contents follows the ionic concentration in plants after a lag period.

The betaine contents in 80 mM plants leaves was high and increased during the first 16 weeks of growth, and then decreased. This fact could be due to the loss of young parts and / or to the decrease of ionic concentration in surviving leaves.

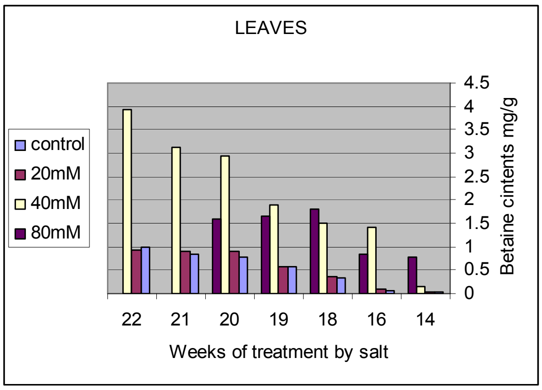
The betaine contents in roots of control and 20 mM plants increased during the experiment and they were very similar. On the contrary, the roots of 40 mM plants had increasing betaine contents and significatively higher that the contents of control plant roots during the first 18 weeks of growth, but the betaine contents were similar to that of control plant roots at the end of the experiment.

The betaine contents in roots of 80 mM plants increased during the first 14 weeks of growth and then decreased and its contents became lower than in control plant roots, showing also much lower a $Na^+ + K^+ + Ca^{2+}$ contents in 80 mM roots than in control ones.

We must conclude from these results, that in *Hyoscyamus aureus* plants the betaine accumulation is not reliable parameter to detect a salinity stress, but free proline contents increases according the salinity conditions.

Table7- Betaine contents in control and treated plants. Expressed in mg/g DW: Date are the average of six analyses Weeks: weeks of treatment

	Control	20 mM	40 mM	80 Mm
Week	**LEAVES**			
14	0.018 ± 0.003	0.023 ± 0.008	0.144 ± 0.021	0.794 ± 0.074
16	0.063 ± 0.010	0.091 ± 0.016	0.401 ± 0.093	0.849 ± 0.062
18	0.333 ± 0.027	0.371 ± 0.033	0.501 ± 0.143	0.800 ± 0.138
19	0.573 ± 0.031	0.574 ± 0.042	0.894 ± 0.176	0.645 ± 0.156
20	0.773 ± 0.062	0.894 ± 0.061	0.932 ± 0.221	0.599 ± 0.189
21	0.849 ± 0.058	0.888 ± 0.063	0.109 ± 0.250	-
22	0.992 ± 0.077	0.915 ± 0.069	0.938 ± 0.307	-
Week	**ROOTS**			
14	0.016 ± 0.006	0.023 ± 0.019	0.110 ± 0.043	0.121 ± 0.022
16	0.045 ± 0.017	0.072 ± 0.043	0.559 ± 0.066	0.610 ± 0.063
18	0.045 ± 0.025	0.070 ± 0.055	0.621 ± 0.075	0.341 ± 0.030
19	0.325 ± 0.043	0.315 ± 0.062	0.615 ± 0.089	0.234 ± 0.041
20	0.380 ± 0.071	0.210 ± 0.094	0.699 ± 0.083	0.210 ± 0.039
21	0.920±0.059	0.810±0.062	0.810±0.077	0.220±0.035
22	0.810±0.088	0.875±0.089	0.881±0.059	0.240±0.068



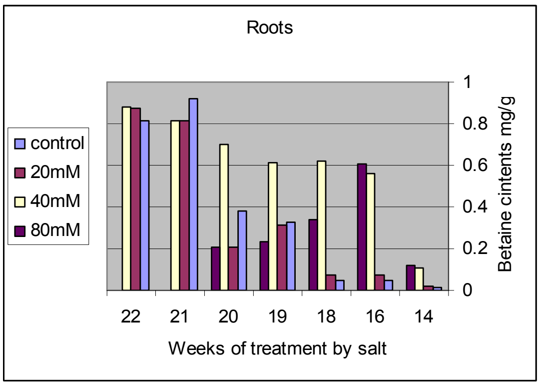


Fig (8): Betaine content of treated and control plants in leaves& roots, expressed in mg/g DW. Data are the average of six analyses

The high proline contents is important in order to solubilise enzymatic proteins in water deficit conditions. This is a salinity stress response, but although the free proline levels in this experiment were higher than 6% to cation values (an amount considered sufficient to protect plants against salinity, (Weimberg]) this response is not enough in *Hyoscyamus aureus* plants to avert the damage and death on 40 and 80 mM plants.

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